Unit 2 Lecture 4

Culturing Microorganisms

Methods of Culturing and Examining Microorganisms - [TheSix I's](#).

Inoculation is the placement of a small portion of the specimen onto media. Once the plate is inoculated it is streaked to **isolate** the bacteria by spreading out the inoculum. Isolation is the processes to obtain individual colonies (pour plate, streak plate, and loop dilution are all isolation methods). The plate is placed into a cabinet that is set a specified temperature for a period of time (usually one day). This is known as **incubation**. The culture is observed or **inspected** for growth. One then compiles all the data in a process called **information gathering.** Finally, based upon the results of biochemical and other tests an **identification** of the organism is made. A pure culture (axenic) results if only a single known species grows. Mixed cultures result if more than one known species grows. And a contaminated culture results if a previously pure or mixed culture has an unwanted organism inoculated onto it. Why is the cultivation of microbes important? It is important to identify microbes that cause disease in order that appropriate therapy is given to the patient.

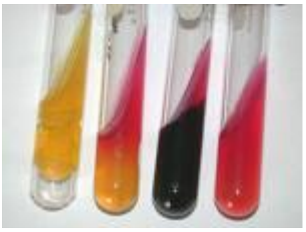
Types of Media

Physical State	Chemical Composition	Functional Type
▪ Liquid ▪ Semisolid ▪ Solid (can be converted to liquid) ▪ Solid (cannot be converted to liquid)	▪ Synthetic (chemically defined) ▪ Nonsynthetic (not chemically defined)	▪ General Purpose ▪ Enriched ▪ Selective ▪ Differential ▪ Anaerobic Growth ▪ Specimen transport ▪ Assay ▪ Enumeration

Media can be differentiated by a number of different aspects. One can describe the media's physical state. **Liquid** media do not solidify at temperatures above freezing. All liquid media are water based. **Semisolid** media are either gelatin based or water based. Semisolid media are used to determine the motility of a microorganism. Most **solid** media can be converted to a liquid if heated. Solid media have a higher percentage of agar in them than do semisolid media. Most media used in a lab are solid media. Some solid media cannot be converted to a liquid when heated. Examples of this type of media include rice grains, cooked meat, and egg based media.

Media can also be described by their chemical composition. A **synthetic** media has all of the constituents chemically defined. **Non-synthetic** media have at least one reagent not chemically defined.

Media can be described by the function they perform. A **general purpose** media is one on which non-fastidious microbes grow. An **enriched** media contains special growth factors added to recover fastidious organisms. A **selectiv**e media incorporates inhibitory agents to select for desired organisms. **Differential** media are designed to show differences among organisms. Often a media can be both selective and differential. An example of this is MacConkey agar that selects for non-fastidious gram negative bacilli and differentiates those that ferment lactose from those that do not. **Anaerobic growth** media (Reducing media) are capable of supporting anaerobic organisms. **Specimen transport** media are designed to maintain organisms from the point of specimen collection until the specimen can be cultured or inoculated on to appropriate plate media. It is very important to choose the appropriate type of transport media when obtaining a specimen from a patient. For example, virus transport media are inhibitory to bacteria and bacterial transport media will not support the growth of viruses. **Assay** media are used in susceptibility testing. Finally, **enumeration** media is used in industry to count the numbers of organisms in a specimen such as food or water supply.



Natural media are produced from one natural source. There is no consistent formula and are also considered non-synthetic. Non-living examples include blood, milk, tissue extracts, and pond water. Living examples include embryonated eggs, laboratory animals, and tissue cultures. These living media are necessary for viruses and special bacteria (*Rickettsia* and *Chlamydia*).

Bacterial Growth

Bacteria like other living organisms have certain growth requirements. The chemical components or elements essential to most living organisms are also required by bacteria. The minimum requirement for growth is a source of carbon and nitrogen, an energy source, water and some ions. Large molecules needed are: C, H, O, N. Positive ions required include Mg^{++}, K^{+}, Na^{+}, Fe^{++}, and Ca^{++}. Negative ions include Cl^{-}, I^{-}, P as PO_4^{-3}, S as SO_4^{-2}. Iron is very important to bacteria, so important that bacteria often secrete special proteins to draw iron from dilute solutions. Oxygen can be a poison for many bacteria.

Groups of molecules compose bacterial cells. Of these water is the most important. It is used as a solvent (70%). Other groups of molecules make up proteins, lipids, carbohydrates, nucleic acid, vitamins, pigments, and inorganic salts.

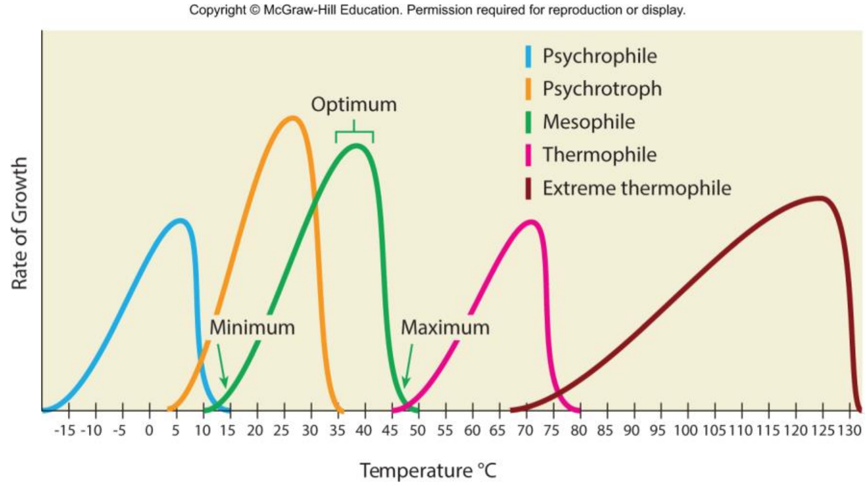
Bacteria can be classified based on the source of carbon for organic synthesis. An **autotroph** or **lithotroph** uses an inorganic source as carbon source (CO_2). **Heterotrophs** recycle organic carbon as a food source. A **parasite** gets organic molecules from a living source whereas **saprophytes** get organic molecules from a non-living source. Bacteria can also be classified based on their source of energy. A **phototroph** is photosynthetic. Therefore it obtains energy from light. A **chemotroph** is chemosynthetic. It obtains energy from oxidation of chemicals. The earliest microbes on the planet were chemoautothrophs.

Environments suitable for growth of microbes.

Microbes live in virtually every environment on this planet, from the deepest depths in the oceans to the highest mountain peaks and every place in between. Microbes live underground and in rocks. They live in environments that are freezing to boiling. They live in highly toxic and polluted environments. Let's explore what these "bugs" need. Water is a basic requirement. Oxygen requirements vary from a strict (obligate) aerobe (elemental oxygen necessary for organism survival) to a strict (obligate) anaerobe organism that is killed by presence of elemental oxygen. The primary colon bacteria are obligate anaerobes. In between these strict environments two one can find the facultative anaerobe which can live without elemental oxygen but prefers to have it present. A microaerophilic organism requires elemental oxygen but only in small amounts.

Temperature requirements

Terms are also used to describe an optimum temperature at which an organism lives. A **Psychrophile** organism grows best at <20°C, some even at <0°C. A **Psychrotroph** can grow slowly in cold temperatures, but have an optimum temperature above 20°C. A **Mesophile** organism grows best between 20-40°C. And a **thermophile** grows best at >45°C. **Extreme thermophile** are terms which indicates survival of heat resistant forms at temperatures of > 100°C. Thermophiles do not cause disease because they 'freeze' at body temperature.



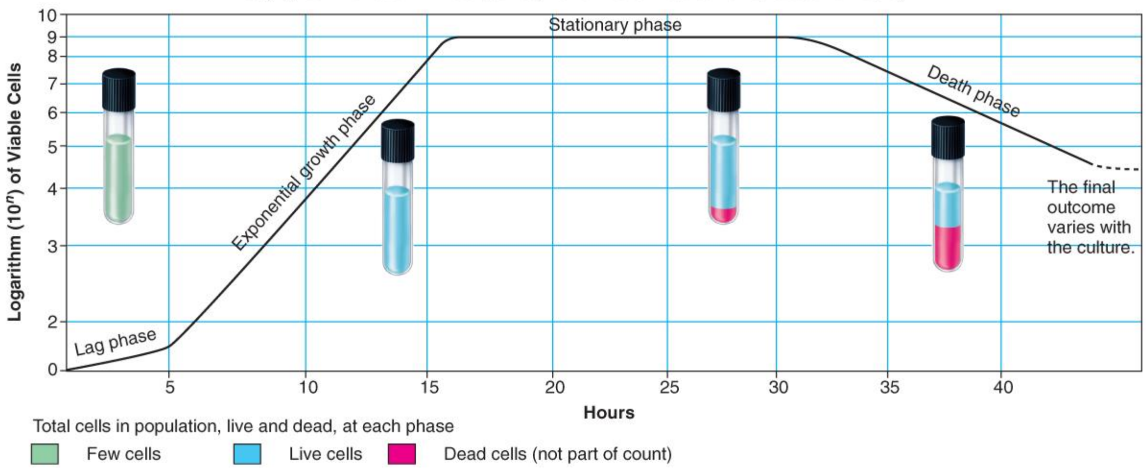
pH requirements

A brief review of the pH scale:

- acidic solutions: 0 - <7.0
- basic solutions: >7.0
- neutral solutions: 7.0

Terms which indicate an optimum pH for bacterial reproduction include acidophile which refers to organisms that prefer an acid environment for growth and alkalophile refers to those organisms that prefer a basic environment for growth. Neutrophiles, organisms that prefer to grow at or around a neutral pH are those that can cause disease. Two terms describe organisms that live in extreme conditions. **Halophiles** prefer an increased salt environment. **Barophiles** prefer an increased atmospheric or hydrostatic pressure. Barophiles are found deep in the ocean.

Bacterial Growth Curve



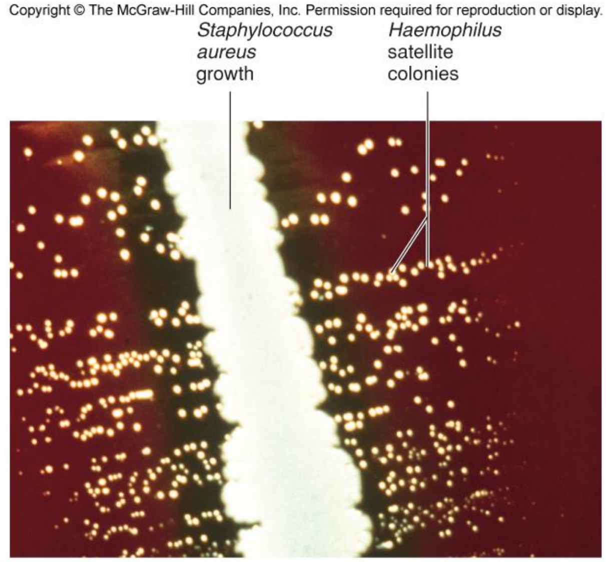
In the **Lag phase** the total number of bacteria remains temporarily unchanged. The cells grow larger than normal size without division. The lag phase can last for an hour to days depending on the species. During the **Log phase** (exponential phase), the population increases rapidly. Cells divide according to their own generation time and remain normal size. *E. coli's* generation time is about twenty minutes whereas some mycobacteria have a generation time of 24-hours. In the **Stationary phase** cell reproduction equals cell death. Reproduction decreases due to decrease of nutrients and the death rate increases due to build up of metabolic wastes. In the last phase of a growth curve, the **Decline (death) phase**, the death rate accelerates, degenerative forms appear. This last phase may be prolonged for days or years.

Evidence of growth in cultures

Growth of microbes can be detected by a number of methods. One of these is the **chemical** changes that occur. Examples include liquefaction of solid media, fermentation of sugars to produce acid and/or gas, or the coagulation of proteins from liquid media. Growth of microbes can also be detected by **visible** changes in liquid media. Make your observations before mixing the tube and look for general turbidity, sedimentation, or surface growth. If your observation is made after mixing, look for fine particles that yield cloudiness or turbidity. Larger particles yield granular appearance. Flakes produce a flocculent appearance and thick heavy solutions are viscid. Visible changes in solid media are easily determined by the development of colonies (growth of bacterial population from a theoretical single cell until visible on a solid media). Characteristics of colonies to look for include form or pattern of growth, elevation, margin, consistency, pigmentation, and odor.

Microbial Interaction

Microbes can interact with other microbes and organisms in a symbiotic or non-symbiotic relationship. In a symbiotic relationship two interactions are possible. **Mutualism** is a reciprocal, obligatory, beneficial relationship to all organisms involved. In **Commensalism**, the organism benefits, but also does not harm other organism i.e. satellitism. In non-symbiotic relationship three situations are possible. First, **Synergism** or **syntrophy** is mutually beneficial but not obligatory to those involved. **Antagonism** or **amensalism** involves competition and inhibition against other organisms detected (antibiosis). The primary reason for this is that organisms are competing for limited resources and some microbes will produce substances that will kill or inhibit other microbes. Finally, **Parasitism** occurs when parasite is independent and lives off the other organism. This situation is most harmful.



The questions you must again ask yourself at this point are, “What is the meaning of life?” How does your answer relate to microbes? What microbial interactions occur so that microbes can 'live'? What do microbes do to other microbes so that they can live?

Bacterial Metabolism

All cells require a constant supply of energy to survive. Metabolism is any controlled chemical activity of the cell that maintains life. A specific type of metabolism is Anabolism, which is activities that create new chemicals for the cell (a building up, biosynthesis of macromolecules necessary for the construction of cellular components, especially proteins). Anabolism requires energy. Catabolism is activities that breakdown chemicals in the cell. These types of reactions release energy. Some bacteria have the property of a system to function in both catabolism and anabolism. This is known as amphibolism.

Enzymes

Enzymes are proteins that serve to catalyze (increase speed) reactions of metabolism. Enzymes require specific conditions of temperature, pH, and osmotic pressure to be activated. They have unique characteristics such as shape, specificity and function. Often enzymes require cofactors, a chemical needed to combine with protein to create an active enzyme. Sometimes they require a coenzyme, an organic cofactor (vitamins), or an activator, an inorganic cofactor (mineral ions) to work. One of the nice things about enzymes is that they are not used up in the reaction. Higher temperatures increase the rate of reactions up to a point. If the temperature rises above that critical point, the enzyme will denature and lose its specific shape.

Enzymes get their names by the chemical reaction that takes place, the substrate the enzyme works on or both plus an "ase", i.e. hydolase, oxidase, lipase, proteinase, ligase. Some terms associated with the location and regularity of enzyme action includes Exoenzymes, (enzymes transported outside of the cell to perform in breaking down a substance) and Endoenzymes (enzymes that function inside the cell. Most enzymes in metabolic pathways are endoenzymes). Constitutive enzymes are always present in relatively constant amounts. Induced enzymes are not currently present and are only produce when its substrate is present. Constitutive and induced enzymes play an important roll in disease. Exoenzymes may be virulence factors such as streptokinase, hyaluronidase, penicillinase, and toxins.

Enzymes play a role in reduction-oxidation (redox) reactions. Redox reactions always occur together. Oxidation is the loss of electrons (LEO). This reaction is important because it provides energy to create ATP. Reduction reaction is the gain of electrons (GER). This reaction is important because it stores energy in molecules.

ATP Synthesis

Energy is needed to fuel the cellular reactions of the cell and is stored in the chemical bonds of ATP. ATP is the energy molecule of the cell, where energy is defined as the capacity to do work or to cause change. Bacteria can produce energy from glucose by fermentation, anaerobic respiration, or aerobic respiration. For obligate anaerobes and facultative organisms, the reaction begins with glycolysis (Embden-Meyerhoff-Parnes Pathway). You can see in the equation below that two ATPs are formed. Four ATP's are formed, but two are used up in the reaction. Anaerobic metabolism is less efficient at energy production than aerobic metabolism.

Glucose --> 2 Pyruvic acids + **2 ATP** + 2$NADH_2$

Anaerobic pyruvic acid conversion equals fermentation. Alcoholic fermentation converts pyruvic acid to ethanol. Lactic fermentation produces lactic acid. Other fermentation produces no additional ATP, but assist in the identification of the organism by looking for acids produced. In obligate anaerobes, succinic, lactic, pyruvic, and other acids are detected by gas chromatography. Biotechnology companies utilize fermentation in creating renewable energy, solvents, and pharmaceuticals.

Aerobic pyruvic acid conversion utilizes the Kreb's cycle or Tricarboxylic Acid (TCA) cycle and produces 2 GPT (ATP). The final reactions in the aerobic cycle occur in the Electron transport system (ETS) or cytochrome oxidase system. It utilizes hydrogen to form water and coenzymes to form ATP. Therefore reaction sequence of ETS in the breakdown of one glucose molecule to two pyruvic acids generates 36 ATPs. The TCA cycle allows the organism to generate a lot more energy than by glycolysis alone.

2 Pyruvic Acid --> 6 CO_2 + 6 H_2O = **36 ATP**

So aerobic cellular metabolism uses both Glycolysis and Kreb's cycle and generates **38 ATP per glucose molecule are formed**. The TCA cycle also supplies key intermediates for the ultimate synthesis of amino acids, lipids, and nucleotides. Why aerobic respiration? Bacteria developed this mechanism, which is used by many organisms, billions of years ago. You can see that aerobic respiration is more efficient and creates more energy in cellular metabolism. Evolution into higher life forms was made possible due to the work of cellular metabolism that bacteria developed.

Strict anaerobes do not use oxygen and so only produce 2 ATP in their cellular cycle. They may use inorganic substances ($SO_4^{=}$, $CO_3^{=}$, NO_3^{-}) as electron carries in production of ATP. This is not a very efficient method of energy production but may be necessary for organism survival.

Take note of one last metabolic reaction; denitrification. Denitrification is important in recycling nitrogen in the biosphere through the breakdown of nitrates.

Where does respiration occur in the cells? In prokaryotes it occurs in the cell membrane whereas in eukaryotes, it occurs in the mitochondria.